Invention Journal of Research Technology in Engineering & Management (IJRTEM)
ISSN: 2455-3689
www.ijrtem.com Volume 2 Issue 7 ǁ July 2018 ǁ PP 87-96

Multivariate regression methods with infrared spectroscopy to detect the falsification of traditional butter

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ABSTRACT : *Recently, food safety and guaranteed of food marks have become more important subjects of foodstuff production and the marketing of processed foods. This paper demonstrates the ability of Mid Infrared spectroscopy coupled with multivariate regression tools to detect vegetable butter (as adulterant) in a binary mixture with traditional cow's butter. Blends of traditional cow's butter with different percentages of vegetable butter were measured using Attenuated Total Reflectance-Fourier Transform Mid Infrared Spectroscopy (ATR-FTMIR). Spectral and reference data were firstly analyzed by principal component analysis (PCA) to check outliers samples; and improve the robustness of the prediction models to be established. Multivariate regression methods as Principal component regression (PCR) and Partial least square regression (PLSR) were used to establish calibration model. Excellent correlation between ATR-FTMIR analysis and studied butter blends was obtained $R^2 = 0.99$; with Root Mean Square Errors of Prediction < 3.04, Limit of Detection 9.12% (By PCR) and 6.06% (by PLSR), and Relative Prediction Errors as low as 3.13.*

KEYWORDS: *Falsification, infrared spectroscopy, multivariate analysis, traditional cow's butter, vegetable butter.*

I. INTRODUCTION

Milk and dairy product consumption is very recommended by most nutrition experts due to their beneficial effects [1], [2]. In Morocco about 20 to 30% of cow's milk produced is still processed individually by farmers. These farmers make traditional Moroccan dairy products such as fermented milks (lben, raib), traditional butter (zabda beldia), and fresh cheese (jben). These products are very popular in Morocco because of their sanitary and refreshing qualities [3], [4]. On the other hand, Falsification of food is causing many problems such as, primarily, the health risks caused by potential food allergens [5], [6]. Consequently, it is very necessary to produce high quality foodstuffs and processed foods. In addition, consumer concern and expectations about food quality has been constantly increasing. Therefore, it becomes necessary for the food industry to have analytical methods for assessing the composition and quality of food and beverages [7], [8]. According to the literature, there are many works that are interested in the falsification and adulteration of food [9], [10], [11].

In this case, many analytical techniques have been developed and used to ensure the quality of dairy products, and especially milk authenticity. For instance, digital color image analysis combined with chemometric methods has been successfully applied to detect adulterations in liquid milks [12] and discriminate adulterated milks from authenticated milks [13], [14]. Also, spectroscopic analyzes as Near InfraRed Spectroscopy (NIRS) has been used in the authenticity of adulterated food [15], [16] and detection of the contents of adulterants in powdered or liquid milk [17], [18], [19]. The spectroscopic method using a Fourier transform infrared (FT-IR) spectrometer equipped with an attenuated total reflection (ATR) accessory [20], [21], has substantial potential as a quantitative technique for such measurements [22], [23]. Fourier Transform Mid Infrared (FTMIR) spectroscopy is a biochemical fingerprinting technique [24]. It can be potentially applied to deliver results with the same accuracy and sensitivity as the reference methods in short time [25]. ATR-FTIR is a fast, cheap, green, simple to use technique with almost no sample pre-treatment and it shows a high selectivity when associated with multivariate analysis tools [26], [27]. Also, Multivariate analysis is often used in spectroscopy to extract used information from complex spectra containing overlapping absorption peaks, interference effects and instrumental artifacts from the data collected. In this context, the actual study presents an application of FTMIR-ATR spectroscopy coupled with multivariate regression methods for quantification and detection analysis of the fraudulent addition of vegetable butter in traditional cow's butter.

This application was considered to develop improved and reliable regression models (PCR and PLSR) which could later be used as a quick and accurate analysis tools for quantifying the actual percentage of vegetable butter in the binary blend with traditional cow's butter.

II. MATERIALS AND METHODS

Samples preparation

In this work, to prepare the adulterated butter samples we used:
- 1 Kilogram of pure and authentic traditional cow's butter was personally taken from farm in Fkih Ben Salah area in central Morocco; and preserved at (- 4°C) until preparation of blends.
- ½ kilogram of vegetable butter was purchased in a local supermarket and preserved at (10°C) until preparation of blends.

Samples were prepared by mixing traditional cow's butter (Ban) with vegetable butter (Bv). Samples with a final mass of 10 g were prepared in different percentages in the 0–40 % weight ratio range of vegetable butter.
All the samples were kept in cold storage (- 4°C) during the nights between the days of measurements. Spectroscopic measurements were taken from samples after they had been brought into equilibrium with the room temperature of 25°C.
There were 58 samples in total, among which 41 samples were randomly taken for establishing principal component analysis (PCA), principal component regression (PCR) and partial least square regression (PLSR) models. Other 17 samples were used for testing the reliability of the models.

Spectra acquisition: FTMIR-ATR spectra were obtained using a PerkinElmer spectrum, Version 10.5.1 equipped with an attenuated total reflectance accessory with DTGS detector, Globar (MIR) Source and KBr Germanium separator, with a resolution of 4 cm^{-1} at 80 scans. Spectra were scanned in the absorbance mode from 4000 to 600 cm^{-1} and the data were handled with PerkinElmer logiciel. About 1g of each binary blend samples of traditional cow's butter and vegetable butter were directly placed, without preparation on an Attenuated Total Reflectance cell provided with a diamond crystal. Analyses were carried out at room temperature (25°C). The background was collected before every sample was measured. Between spectra, the ATR plate was cleaned by ethanol.

Data pre-processing procedures and software: In this study, a series of pre-processing elaborations were tested on the spectral data prior to the multivariate calibration. The Savitzky–Golay [28] and Norris gap [29] algorithms were tested for data derivatisation. Standard normal variate (SNV) and multiple scatter correction (MSC) [30] were also tested.

Multivariate regression analysis: The multivariate analysis was performed by the following multivariate techniques: principal component analysis (PCA), Principal Component Regression (PCR) and Partial least squares regression (PLSR). In fact, the multivariate regression methods like principal component regression (PCR) and partial least squares regression (PLSR) enjoy large popularity in a wide range of fields [31], [32], [33]. In this study, we used PCA, PCR and PLSR as methods for quantification of traditional cow's butter adulterations according to FTMIR-ATR analysis. The pre-treatment procedures and all chemometric models (PCA, PCR and PLSR) were performed using The Unscrambler X version 10.2 (CAMO, Oslo, Norway).

III. RESULTS AND DISCUSSION

Spectra analysis: FTMIR-ATR spectra of 58 samples of the studied binary mixtures were recorded and divided in two sets: a calibration set of 41 samples and an external validation set of 17 samples. One spectrum is the average of 80 scans of the same sample of adulterated butter. The average spectra of all considered samples in calibration set are presented in

Fig.1.

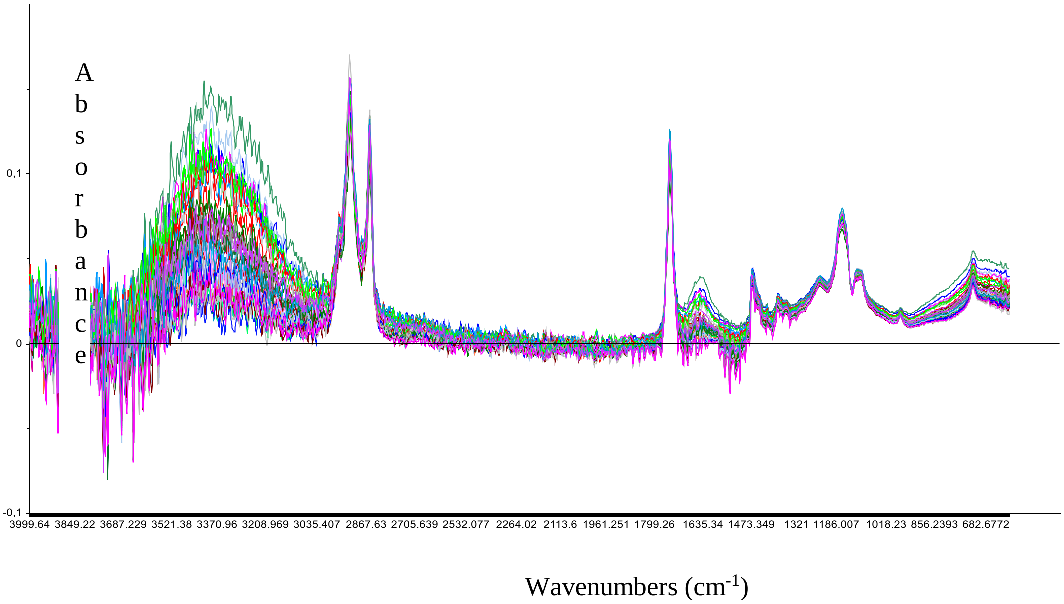


Fig.1. FTMIR-ATR spectra of the binary mixture (traditional Cow's butter – Vegetable butter: Ban-Bv) samples of calibration set in the 0–40% weight ratio range.

In **Fig.1**, the obtained spectra are dominated by the significant bands of water are clearly visible in the studied butters spectra at 3400 cm^{-1}. The band of aromatic ring stretch of lignin should appear at 1604 cm^{-1}. However, this region was obscured by the strong water deformation band centered at 1638 cm^{-1}. The typical infrared pattern of sugar is observed in the region 1200 - 900 cm^{-1}. The two small bands at 2927 cm^{-1} and 2856 cm^{-1} are characteristic of fatty acids.
According to

Fig.1, the FTMIR-ATR spectra obtained of the adulterated samples to be similar. In this case, multivariate analysis appeared to be ideal to provide an effective solution, as they allow extracting of unspecific analytical information from the full-spectra or large regions of them.

PCA Modeling: Principal component analysis was carried out to detect the presence of any spectral outliers in the spectral data, prior to develop two prediction models using PCR and PLS regression.

Fig.2 shows the score plot obtained by PCA model in calibration set of adulterated butters.

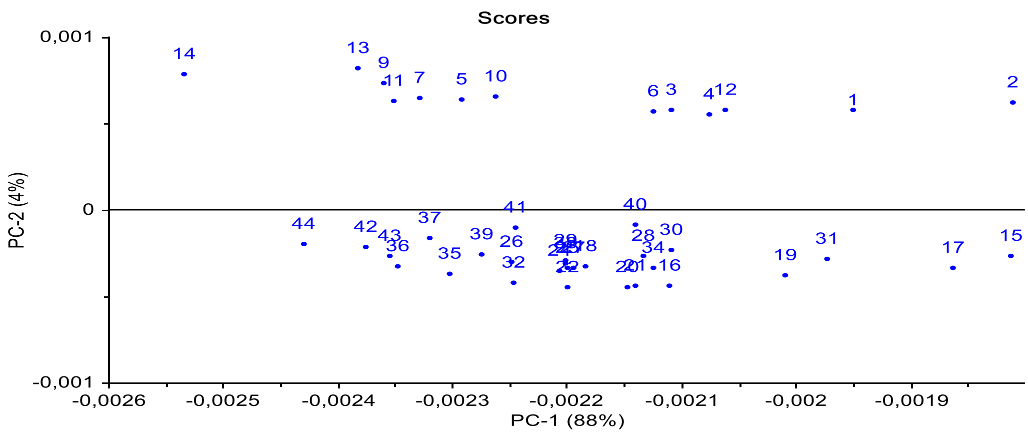
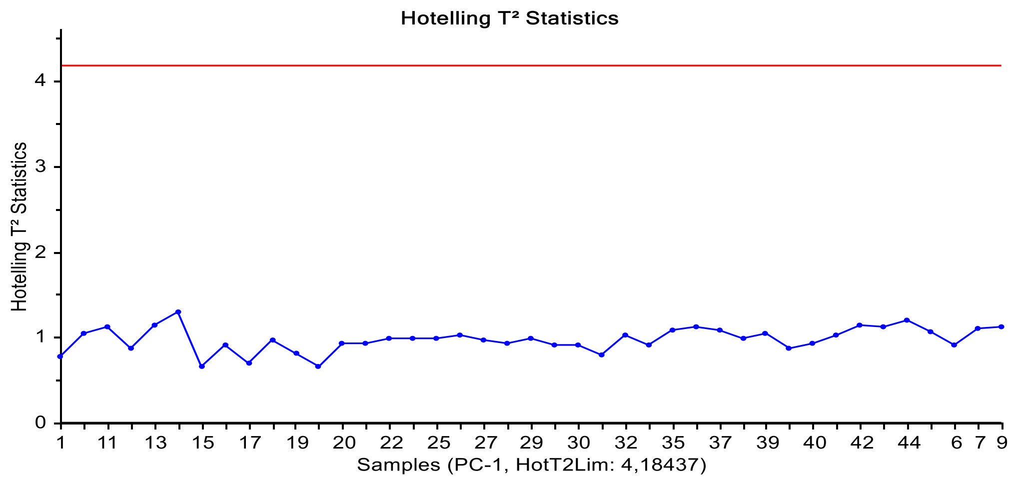


Fig.2. PC1 / PC2 Score plot by PCA analysis on the calibration set of binary mixtures (Traditional Cow's Butter-Vegetable butter) samples.

To verify the true nature of the all samples, we have looked to the Hoteling T² statistics plot (Fig.3).). It is an alternative to plotting sample leverages. The plot displays the Hotelling T² statistic for each sample as a line plot. The associated critical limit (with a default p value of 5%) is displayed as a red line. In fact, the Hotelling T² statistic has a linear relationship to the leverage for a given sample. Its critical limit is based on an F-test. Use it to identify outliers or detect situations where a process is operating outside normal conditions. According to

Fig.3 there isn't any outlier



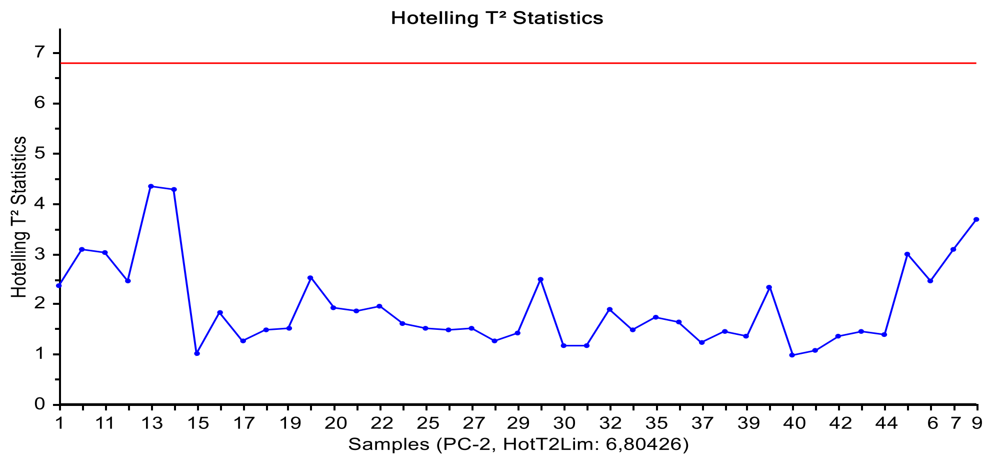


Fig.3.PC1 / PC2 Hotelling T² statistics of calibration set.

PCR and PLSR Modeling: In general, the modeling consists of two steps: The first is calibration (construction of model), where data characteristics (Calibration and internal validation samples) are investigated to find a model for their behavior; and the second is External validation, where data that did not participate in the calibration step (external validation samples) are used to evaluate the model adequacy and capability. The PCR and PLSR models were evaluated using coefficient of determination (R^2) in calibration, root-mean-square error of calibration (RMSEC) and cross validation (RMSECV). Root mean square error of cross-validation (RMSECV), recovery percentage and coefficient of determination (R^2) were used as parameters to determine appropriate number of principal components (PCs of PCR) or of latent variables (LVs of PLSR) [34], *[35]*.

PCR / Calibration : The PCR model is built by considering the all spectra range 4000-600 cm^{-1} with X as variable and the Y variables is associated to percentages in the 0–40 % weight ratio range of vegetable butter in adulterated butters. Then, the model is validated (internal validation) by full cross validation. The obtained statistical parameters RMSEC, RMSEV and R^2 are summarized in **Fig.4**. The coefficient of determination R^2 of

0.99, RMSEC of 1.409 and RMSEV of 1.733, could be considered satisfactory. And three PCs are necessary to have a good PCR performance, with explained variances above 99% (**Fig.5**).

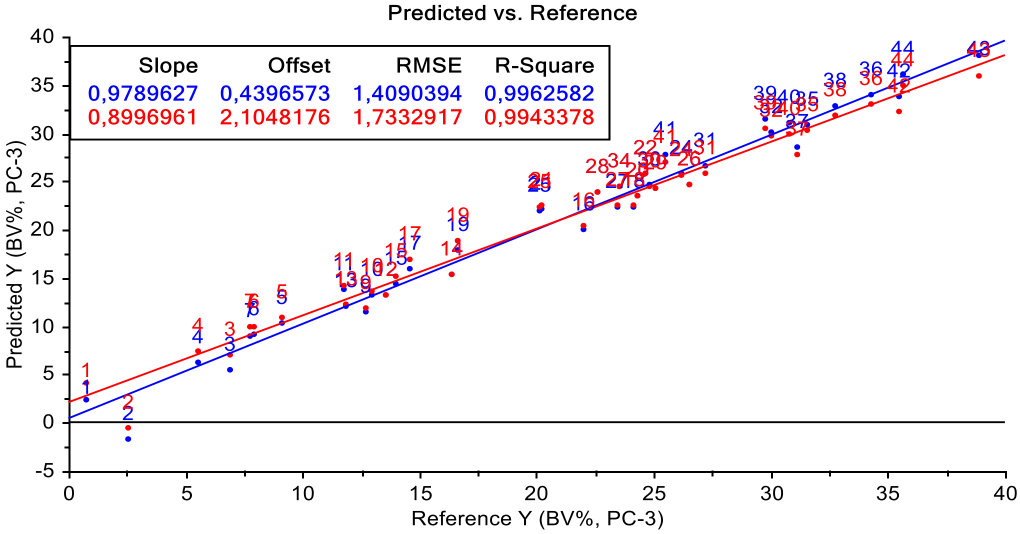


Fig.4. Measured vs. Predicted values for vegetable butter in the studied binary mixtures obtained from the final PCR model developed from the FTMIR-ATR spectra.

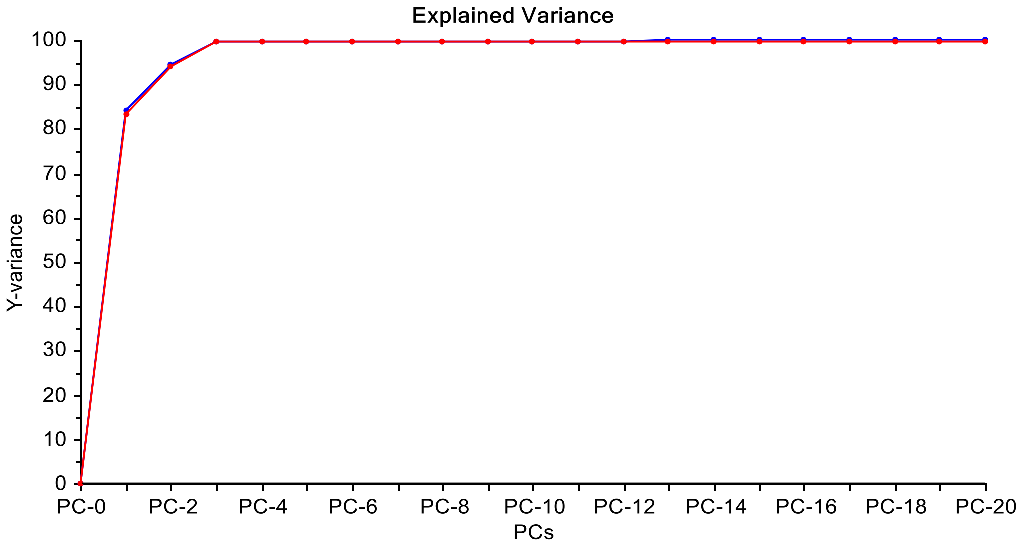


Fig.5. Plot of explained variance of factors describing PCR model.

PCR /External validation: The PCR model was applied to a group of external samples; the results are listed in Table 1 and the results are shown in Fig.6. The deviations of prediction of vegetable butter composition in the blend samples by FTMIR-ATR spectroscopy were between 1.53 and 1.86, which were very satisfied (Table 1).
Fig.6 shows the PCR model reconstructed by external validation samples, following the same previous pre-treatments. This PCR model correlates the « actual » and « predicted » values of vegetable butter percentages obtained from FTMIR-ATR spectra. The difference between the actual and the predicted percentage is relatively small. Figures of merit of the calibration graphs are summarized in **Fig.6**. As can be seen, PCR model offered good values for the different multivariate parameters, with limit of detection 9.12%.

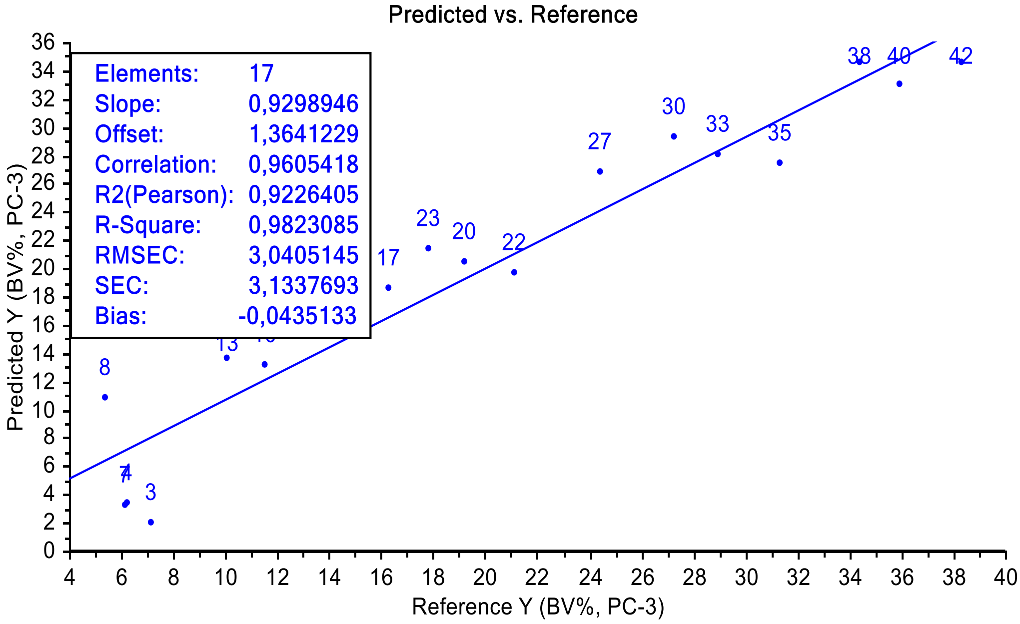


Fig.6. Measured vs. Predicted values for vegetable butter in binary mixtures traditional cow's butter-vegetable butter obtained from PCR

TABLE 1 Prediction results of vegetable butter content (%) in the adulterated butter samples by FTMIR-ATR

Spectroscopy coupled with PCR.

SAMPLES OF EXTERNAL VALIDATION	PREDICTED	DEVIATION	REFERENCE
S1	14.1683	1.6599	10.0757
S2	22.5435	1.8606	16.2701
S3	21.9688	1.7318	21.1313
S4	26.9783	1.5300	24.3921
S5	5.5033	1.7611	7.1149
S6	28.4487	1.7463	27.2244
S7	29.4141	1.6352	31.3394
S8	31.2706	1.7439	34.4086
S9	6.5110	1.7586	6.2373
S10	34.0301	1.6296	38.2864
S11	6.4568	1.7608	6.1125
S12	23.4824	1.7982	17.8186
S13	30.1872	1.7179	28.9368
S14	12.5185	1.8228	5.3596
S15	23.4331	1.5553	19.1766
S16	13.2946	1.6597	11.5159
S17	31.1651	1.5854	35.9402

PLSR / Calibration: Likewise, the PLSR model is built by considering the all spectra range 4000-600 cm^{-1} with X as variable and the Y variables is associated to vegetable butter percentages in adulterated butters. The PLSR model is validated by full cross validation. The obtained statistical parameters RMSEC, RMSEV and R^2 are summarized in **Fig.7**.

The coefficient of determination R^2 of 0.99, RMSEC of 1.115 and RMSEV of 1.602, could be considered satisfactory. And three VLs are necessary to have a good PLSR performance, with explained variances above 99%.

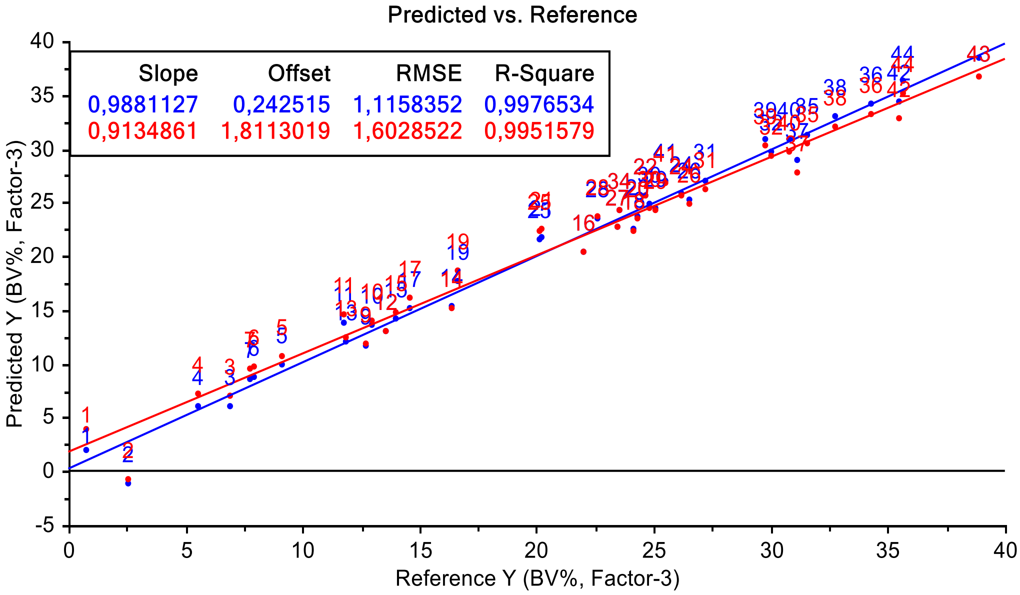


Fig.7. Measured vs. Predicted values for vegetable butter in the studied binary mixtures obtained from the final PLSR model developed from the FTMIR-ATR spectra.

PLSR / External validation: The PLSR model was applied to a group of external samples, the results are listed in **Table 2** and the results are shown in **Fig.8**. The deviations of prediction of vegetable butter composition in the blend samples by FTMIR-ATR spectroscopy were between 1.41 and 1.72, which were very satisfied (**Table 2**). **Fig.8** shows the PLSR model reconstructed by external validation samples, following the same previous pre-treatments. This PLSR model correlates the « actual » and « predicted » values of vegetable butter percentages obtained from FTMIR-ATR spectra. The difference between the actual and the predicted percentage is relatively small. Figures of merit of the calibration graphs are summarized in **Fig.8**. As can be seen, PLSR model offered good values for the different multivariate parameters, with limit of detection 6.06%.

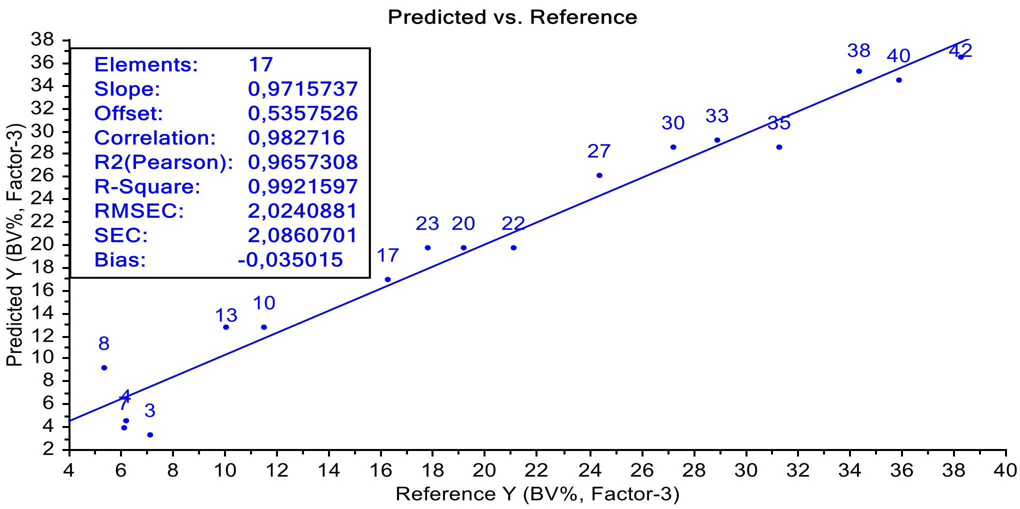


Fig.8. Measured vs. Predicted values for vegetable butter in binary mixtures traditional cow's butter-vegetable butter obtained from PLSR with external validation set.

TABLE 2 PREDICTION RESULTS OF VEGETABLE BUTTER CONTENT (%) IN THE ADULTERATED BUTTER SAMPLES BY FTMIR-ATR SPECTROSCOPY COUPLED WITH PLSR.

SAMPLES OF EXTERNAL VALIDATION	PREDICTED	DEVIATION	REFERENCE
S1	14.4295	1.5354	10.0757
S2	22.2062	1.7202	16.2701
S3	22.0208	1.6024	21.1313
S4	26.8312	1.4150	24.3921
S5	5.3889	1.6300	7.1149
S6	28.6222	1.6186	27.2244
S7	29.4151	1.5121	31.3394
S8	31.0838	1.6186	34.4086
S9	6.6806	1.6272	6.2373
S10	34.2448	1.5057	38.2864
S11	6.2320	1.6298	6.1125
S12	23.3112	1.6633	17.8186
S13	30.4499	1.5879	28.9368
S14	12.7658	1.6858	5.3596
S15	23.6150	1.5316	19.1766
S16	13.2946	1.6597	11.5159
S17	30.8700	1.4807	35.9402

IV. CONCLUSION

Multivariate regression analysis associated with Fourier Transform Mid Infrared Spectroscopy is found to be a successful technique to detect and predict the percentage of vegetable butter as adulterant, in traditional cow's butter. In fact, the both PCR and PLSR models obtained from transformed infrared spectra gave excellent statistical parameters: correlation coefficients of 0.99 and root mean square errors of prediction (RMSEP) less than 3.04. Finally, we arrived to develop a new application of the FTMIR-ATR associated with regression tools (PCR and PLSR) as a rapid, inexpensive and non destructive authenticity and falsification measuring technique, useful to determine the percentage of vegetable butter in the binary mixture with traditional cow's butter. This approach can be used in dairy industry for the reliable, cheap and fast quality control and routine analysis.

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W. Terouzi (2018). Multivariate regression methods with infrared spectroscopy to detect the falsification of traditional butter. Invention Journal of Research Technology in Engineering & Management (IJRTEM), 2(7), 87-96. Retrieved July 25, 2018, from www.ijrtem.com.